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(54) **INTEGRATED MICROBIAL COLLECTOR**

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G01N 21/00 (2006.01)

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C12M 1/34 (2006.01)

(52) **U.S. Cl.** **356/335**; 356/336; 356/338; 435/288.7; 435/34

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See application file for complete search history.

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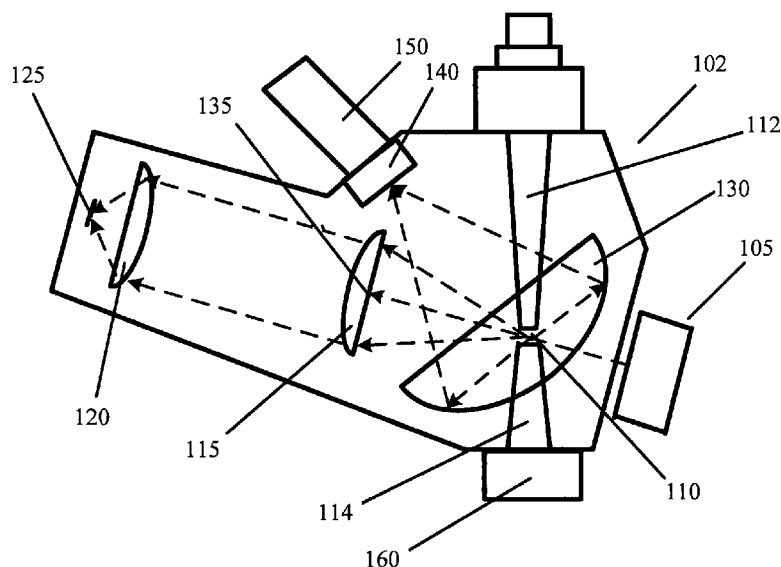
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(57) **ABSTRACT**

A system for real-time sizing of fluid-borne particles is disclosed. The system further determines, in real time, whether the detected particles are biological or non-biological. As the fluid is being tested, it is exposed to a microbe collection filter which is cultured to determine the type of microbes present in the fluid being tested.

17 Claims, 6 Drawing Sheets



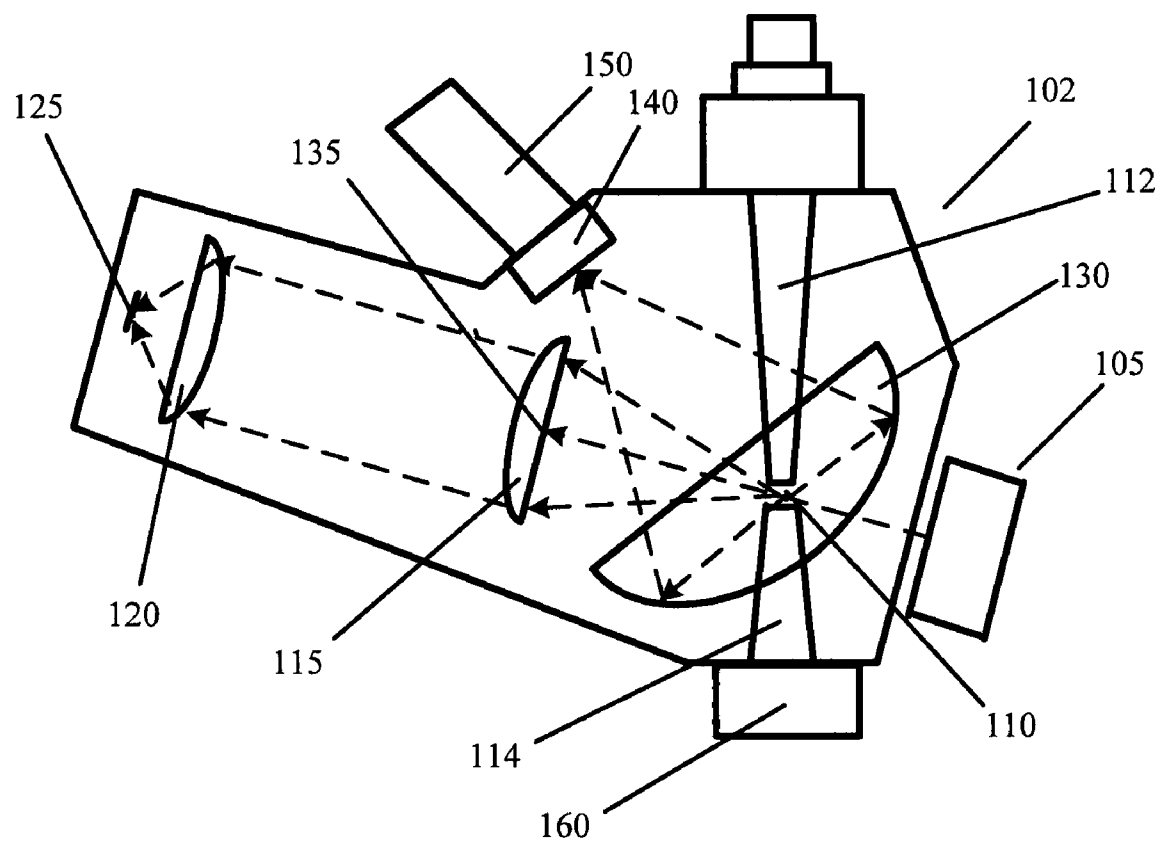


Fig. 1

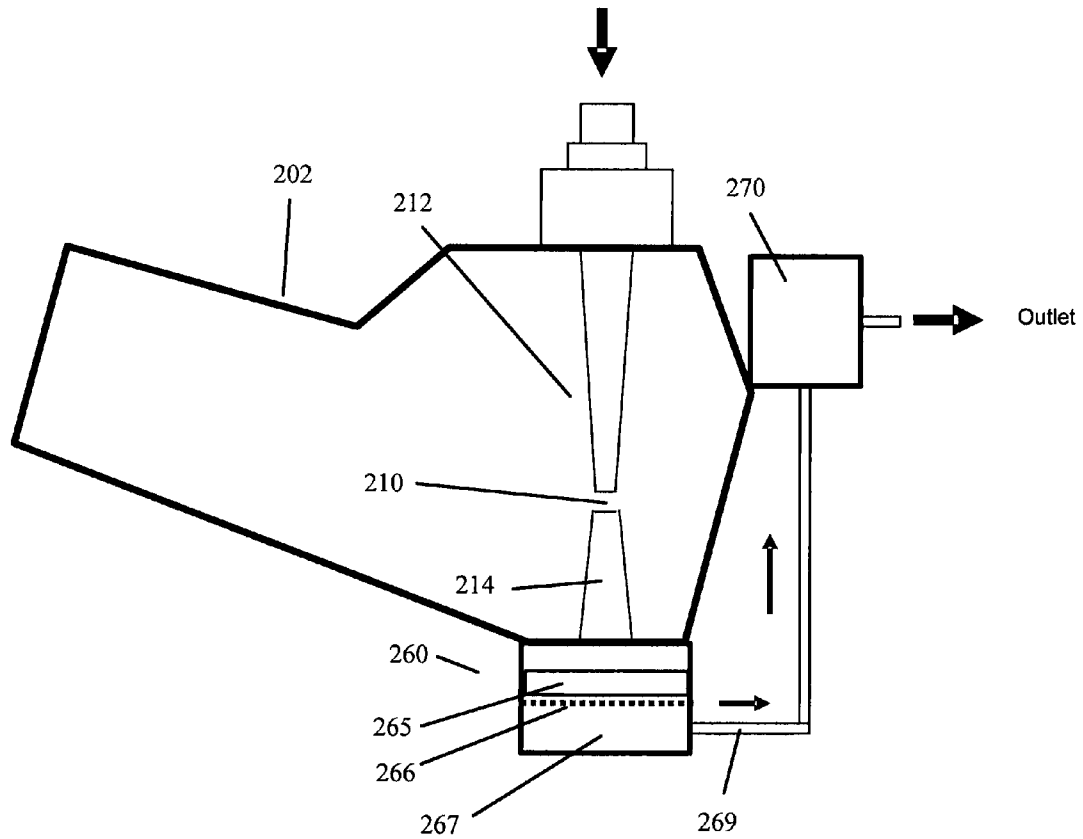


Fig. 2

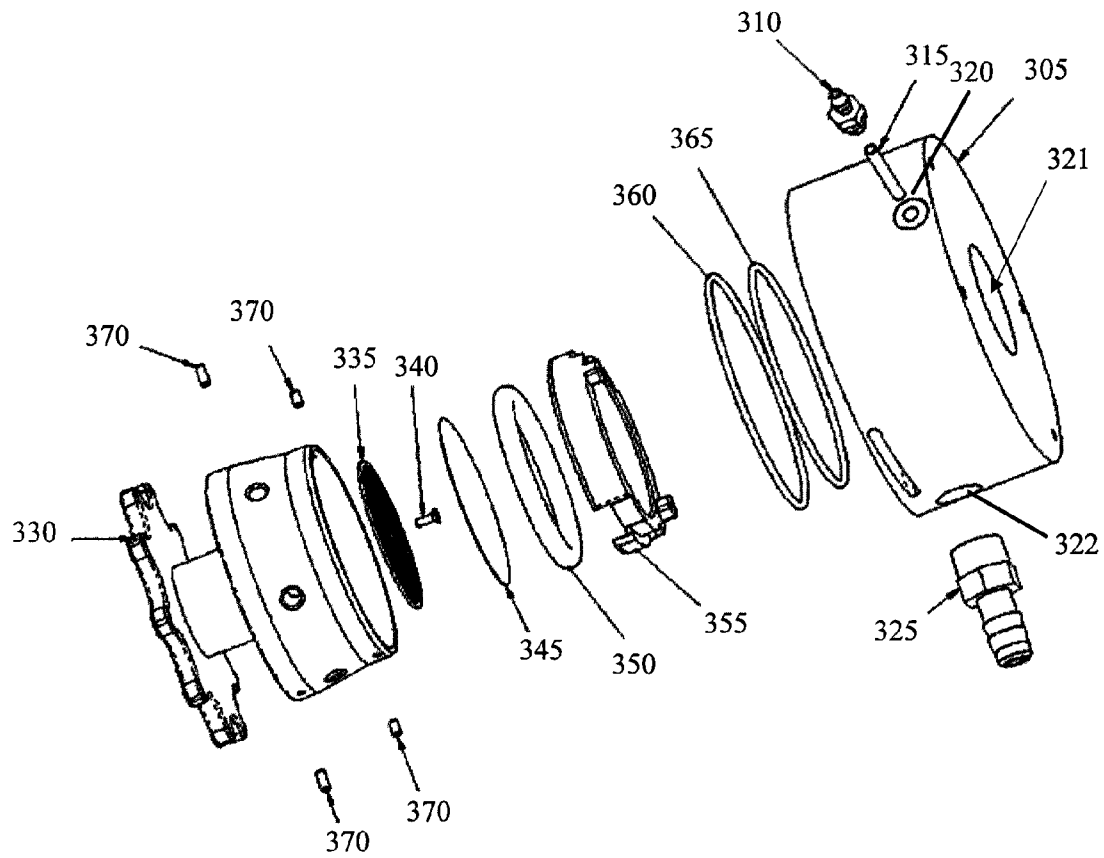


Fig. 3

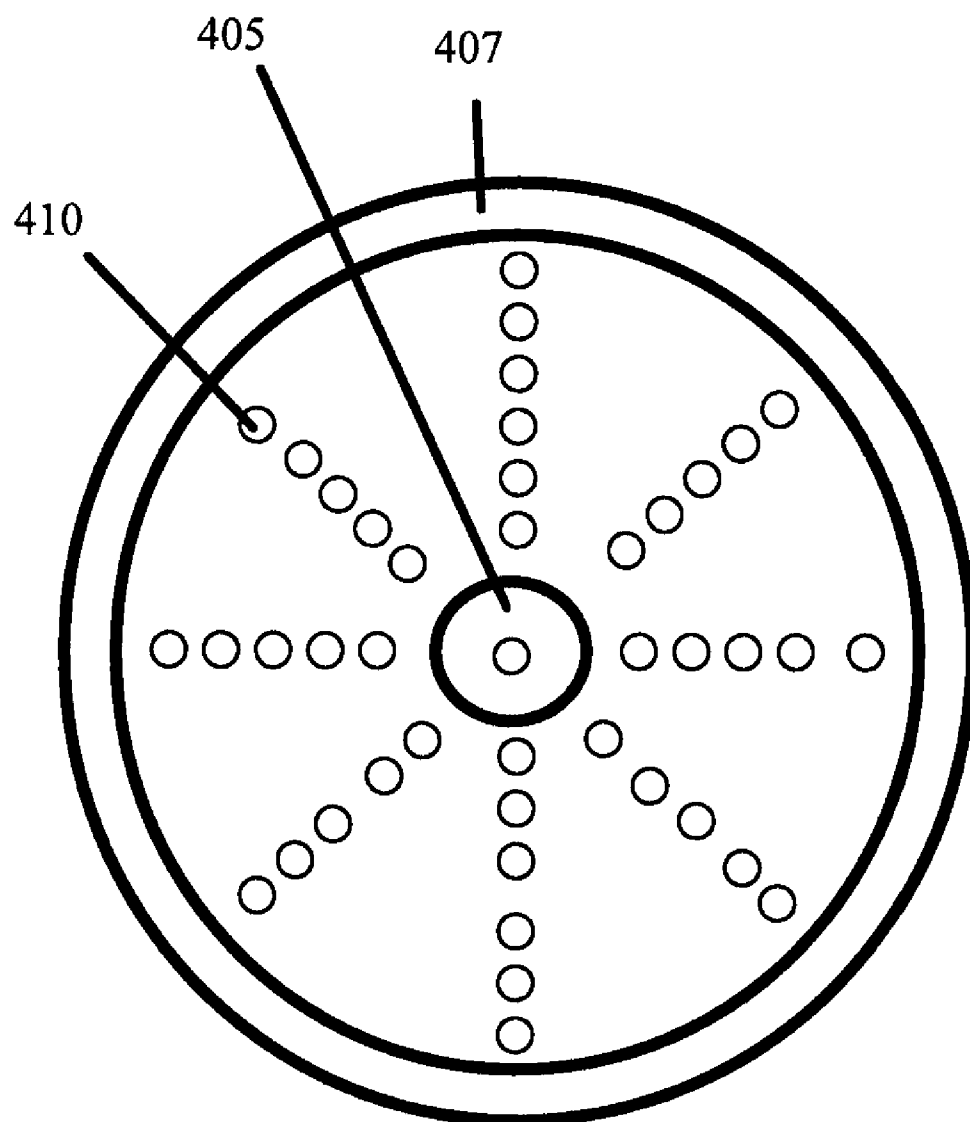


Fig. 4

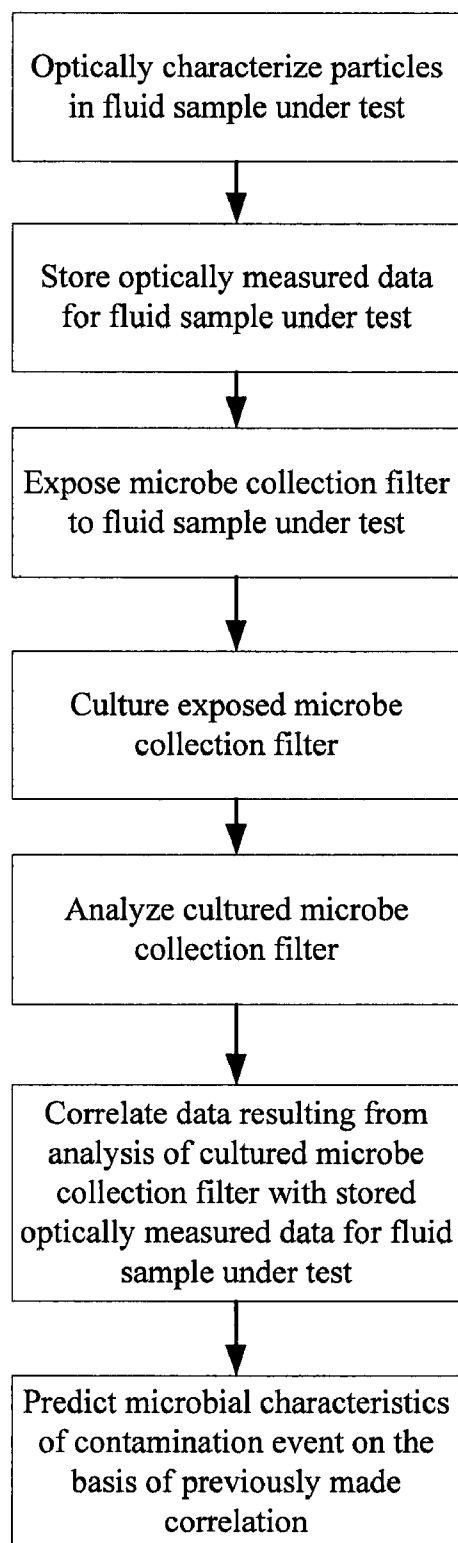


Fig. 5

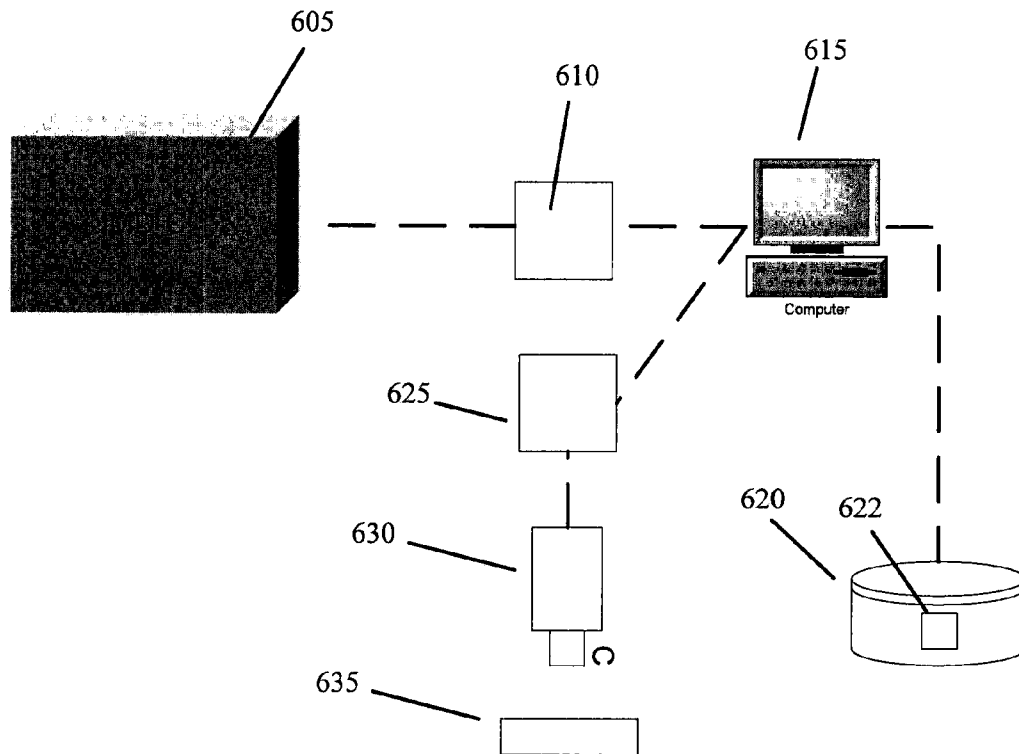


Fig. 6

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INTEGRATED MICROBIAL COLLECTOR**CROSS-REFERENCE TO RELATED
APPLICATION**

This application claims priority to U.S. Provisional Application entitled "Integrated Microbial Collector," having Ser. No. 61/138,878, filed Dec. 18, 2008, the contents of which are entirely incorporated herein by reference.

FIELD OF INVENTION

The present invention relates to generally to a system and method for detecting airborne or liquid-borne particles (generally, fluid-borne particles), and more particularly to a system and method for detecting airborne or liquid-borne particles, determining the size of the particles, determining the particles' status as biological or inert, and classifying the type of biologic particle detected.

BACKGROUND OF INVENTION

A variety of manufacturing environments require strict control over the presence of foreign debris in the air. Semiconductor manufacturing, for example, has long required "clean-rooms" that use extensive air filtering to reduce the number and size of particles in the air to some acceptable level. Other manufacturing environments have similar but distinct requirements. For example, in pharmaceutical or medical device manufacturing environments it is critical to control not only the number of particles in the air, but minimization of biologic particles is of particular importance. Microbial contamination, for example, can render an entire batch of pharmaceutical product unusable leading to significant monetary losses in the manufacturing process. Additionally, it is advantageous to have instantaneous detection of contamination events, including instantaneous information about whether a contamination event is biologic or non-biologic, during the manufacturing process for pharmaceuticals or medical devices.

A variety of systems and methods exist that provide instantaneous detection of fluid borne particles. For example, certain detectors have been designed to detect fluid borne particles and provide warning when the number of particles within an air sample exceeds a predetermined minimum value. Exemplary devices are described in U.S. Pat. Nos. 5,646,597, 5,969,622, 5,986,555, 6,008,729, and 6,087,947, all to Hamburger et al. These detectors all involve direction of a light beam through a sample of environmental air such that part of the beam will be scattered by any particles in the air, a beam blocking device for transmitting only light scattered in a predetermined angular range corresponding to the predetermined allergen size range, and a detector for detecting the transmitted light. An alarm is actuated if the light detected at the detector is above a predetermined level.

Additionally, systems and methods exist that instantaneously determine whether detected particles are biologic or inert. For example, U.S. Pat. No. 7,430,046 to Jiang et al., discloses systems and methods for simultaneously measuring particle size by use of Mie scattering and determining whether the measured particles are biologic or inert by detecting fluorescence excited in certain biological chemicals present in the measured particles.

Although fluorescence analysis can, in certain cases, be used to determine the type of biologic particle detected, i.e., the type of organism, it would be advantageous to have additional systems and methods that could simultaneously collect

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information on particle size, whether a particle is biologic or non-biologic, and the type of biologic particle that has been detected.

SUMMARY OF THE INVENTION

Embodiments of the invention continuously sample fluid containing particles from an environment to be monitored. Sampled fluid is passed through a sampling area, where it is exposed to light. Light scattered by the particles in the fluid is detected and used to determine the size of the particles. Light emitted by fluorescence from the particles is also detected and used to determine whether the particles are biological or non-biological. A filter containing a gas-permeable substance to which microbes adhere, (i.e., a microbe collection filter) is placed after the sampling area, such that the microbe collection filter is placed into the flow of the fluid being measured. In certain embodiments, the filter is situated atop a circular support plate including perforations allowing fluid to flow through the plate. The microbe collection filter collects biological particles from at least a portion of the flow of fluid. After optical measurements have been performed for a predetermined amount of time, and microbe collection filter has been exposed, the filter is removed, microbial nutrients and/or water are added if necessary, and the filter is incubated and examined for the growth of organisms according to any of a number of means, e.g., colony counting, observing the macroscopic appearance of the growth patterns of microbes on the filter, microscope observation of the microbes or chemical testing for the metabolic by-products of microbial growth.

In one embodiment, a microbial detection and identification system is described. The system includes a sampling area including a fluid, a light source, a first optical detector and a second optical detector. The system further includes a microbe collection filter in fluid communication with the sampling area. The light source illuminates particles in the sampling area, the first detector detects light scattered into a predetermined angular range by particles of a predetermined size, and the second detector measures light emitted by fluorescence from illuminated biological particles in the sample area. Additionally, the fluid is exposed to the microbe collection filter causing particles in the fluid to adhere to the microbe collection filter.

In certain embodiments, a first optical system directs scattered light from the sampling area to the first detector and a second optical system that directs fluorescence light from the sampling area to the second detector. In certain embodiments, the second optical system includes a long-pass filter that selectively transmits light having a wavelength of light emitted by fluorescence by illuminated particles in the sample area. In certain embodiments, an ellipsoidal reflector is included having a first focus at the sampling area and a second focus near the second detector.

In certain embodiments, the long-pass optical filter comprises two long-pass reflective filters in series. In certain embodiments, the light source comprises an LED or a diode laser, and the light source emits at a wavelength of approximately between 350 nm and 410 nm. In certain embodiments, the microbe collection filter includes a first surface, a second surface, and a gas permeable interior allowing the fluid to flow into the first surface, and out of the second surface. In certain embodiments, the microbe collection filter is gelatin.

In some embodiments, the fluid is air, and additional embodiments include a blower in fluid communication with the sampling area, where the blower supplies negative pressure to the sampling area thereby drawing environmental air into the sampling area and evacuating air from the sampling

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area as it is optically measured. In certain embodiments, the blower is in fluid communication with the microbe collection filter, and air is drawn through the microbe collection filter after it is optically measured.

Certain embodiments recite a method of detecting microbial contamination in a fluid. The method involves illuminating a fluid with a light source, detecting particles of a predetermined size range present in the fluid by measuring light scattered by illuminated particles into a predetermined range of angles, classifying particles in the fluid as biological or non-biological by measuring fluorescent light emitted from illuminated particles, and exposing a microbe collection filter to the fluid.

Certain embodiments include storing data related to the detection of particles of a predetermined size range and classification of particles as biological or non-biological for further analysis. Additional embodiments include culturing the exposed microbe collection filter. Other embodiments include analyzing the cultured exposed microbe collection filter to determine the types of microbes present on the microbe collection filter at the time of exposure.

Certain embodiments include analyzing the cultured exposed microbe collection filter comprises one or more of: colony counting, observing the macroscopic appearance of the growth patterns of microbes on the cultured exposed microbe collection filter, microscope observation of microbes on cultured exposed microbe collection filter, and chemical testing for the metabolic by-products of microbial growth present on the cultured exposed microbe collection filter. Other embodiments include storing data related to the detection of particles of a predetermined size range and classification of particles as biological or non-biological for further analysis. Certain embodiments include correlating the determination of the types of microbes present on the microbe collection filter at the time of exposure with stored data related to size of particles present in the fluid at the time of exposure and the status of the particles as biological or non-biological.

Certain embodiments include a method of characterizing a contamination event. The method involves illuminating a first fluid with a light source, detecting particles of a predetermined size range present in the first fluid by measuring light scattered by illuminated particles into a predetermined range of angles, and classifying particles in the first fluid as biological or non-biological by measuring fluorescent light emitted from illuminated particles. The method also includes, storing data related to a scattering and fluorescence characteristics of particles in the first fluid, exposing a microbe collection filter to the first fluid, culturing the microbe collection filter and analyzing the cultured exposed microbe collection filter to determine the types of microbes present on the microbe collection filter at the time of exposure with stored data related to the scattering and fluorescence characteristics of particles in the first fluid, optically detecting scattering and fluorescence characteristics of particles in a second fluid, and comparing the detected scattering and fluorescence characteristics in the second fluid with the stored data related to the scattering and fluorescence characteristics of particles in the first fluid. Other embodiments are directed to a method where on the basis of the comparison, determining that microbes present in the first fluid are likely present in the second fluid.

Advantages of the invention include the ability to perform instantaneous, simultaneous particle sizing and detection of biological or non-biological organisms. Additional advantages

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include the ability to determine the type of biological particle detected and correlate data on the type of organism detected with the real-time particle data to fully characterize a contamination event. Additional advantages include the possibility of predicting the type of microbial contamination associated with future events on the basis of past correlations between optical and growth-medium measurements of particles.

BRIEF DESCRIPTION OF THE DRAWINGS

Implementations of the invention will become more apparent from the detailed description set forth below when taken in conjunction with the drawings, in which like elements bear like reference numerals.

FIG. 1 is a schematic diagram of a system for instantaneous and simultaneous particle sizing and biological detection according to an embodiment of the invention.

FIG. 2 is a schematic diagram showing the microbial collector components of a particle sizing and biological detection system according to an embodiment of the invention in additional detail.

FIG. 3 is an exploded drawing showing an assembly for holding a microbe collection filter according to an embodiment of the invention.

FIG. 4 is a plan view of an exemplary filter support plate suitable for use in conjunction with an embodiment of the invention.

FIG. 5 is a schematic flowchart illustrating steps of a method of microbial detection and analysis according to an embodiment of the invention.

FIG. 6 is a schematic block diagram of a computer-automated machine vision and analysis system according to an embodiment of the invention.

DETAILED DESCRIPTION OF THE INVENTION

This invention is described in preferred embodiments in the following description with reference to the Figures, in which like numbers represent the same or similar elements. Reference throughout this specification to "one embodiment," "an embodiment," or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment," "in an embodiment," and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

The described features, structures, or characteristics of the invention may be combined in any suitable manner in one or more embodiments. In the following description, numerous specific details are recited to provide a thorough understanding of embodiments of the invention. One skilled in the relevant art will recognize, however, that the invention may be practiced without one or more of the specific details, or with other methods, components, materials, and so forth. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the invention.

The schematic flow charts included are generally set forth as logical flow chart diagrams. As such, the depicted order and labeled steps are indicative of one embodiment of the presented method. Other steps and methods may be conceived that are equivalent in function, logic, or effect to one or more steps, or portions thereof, of the illustrated method. Additionally, the format and symbols employed are provided to explain the logical steps of the method and are understood

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not to limit the scope of the method. Although various arrow types and line types may be employed in the flow chart diagrams, they are understood not to limit the scope of the corresponding method. Indeed, some arrows or other connectors may be used to indicate only the logical flow of the method. For instance, an arrow may indicate a waiting or monitoring period of unspecified duration between enumerated steps of the depicted method. Additionally, the order in which a particular method occurs may or may not strictly adhere to the order of the corresponding steps shown.

FIG. 1 shows a system for instantaneous particle sizing and biological particle detection according to an embodiment of the invention. The system of FIG. 1 includes a housing 102, which serves to seal and/or isolate the interior components of the system from contamination from the surrounding air and interference from light sources in the environment of the system.

The system of FIG. 1 further includes a light source 105. In one embodiment, light source 105 produces an output having a wavelength between 270 nm and 410 nm. In one embodiment, light source 105 produces an output having a wavelength between 350 nm and 410 nm. In one embodiment, light source 105 produces an output having a wavelength of about 405 nm. The spectral characteristics of light source 105 are such that light emitted by light source 105 is capable of undergoing Mie scattering when interacting with particles of a size range of interest. Additionally, light source 105 is selected to have a wavelength capable of exciting intrinsic fluorescence from metabolites inside microbes and other biological particles. A wavelength of about 270 nm to 410 nm is chosen based on the observation that microbes and biological particles contain at least one of a number of primary metabolites that exhibit fluorescence: tryptophan, which normally fluoresces at excitation wavelengths of about 270 nm, with a range of about 220 nm to about 300 nm; nicotinamide adenine dinucleotide (NADH), which normally fluoresces at excitation wavelengths of about 240 nm, with a range of about 320 nm to about 420 nm; and riboflavin, which normally fluoresces at wavelengths of about 200 nm, with a range of between 320 nm to about 420 nm. In the case of bacterial endospores, dipicolinic acid (DPA) normally fluoresces at excitation wavelengths of about 400 nm, with a range of about 320 nm to about 420 nm. A light source having a wavelength output of about 350 nm to about 410 nm ensures excitation of two of the three aforesaid primary metabolites: NADH and riboflavin, as well as DPA. Selection of this wavelength band allows for fluorescence to be generated and detected in bio-particles, but excludes excitation of non-biological sources of fluorescence such as diesel engine exhaust and other inert particles such as dust or baby powder.

Light source 105 can be a laser such as a diode laser, an LED or a spectrally filtered broadband source such as a lamp. Light source 105 can optionally include collimating or beam shaping optics to produce a substantially collimated output and/or an output having a transverse beam profile that is flat, in terms of power. Optionally, light source 105 includes an optical fiber that delivers light to the vicinity of the other elements of the system of FIG. 1. When an optical fiber is used to deliver light from a remotely situated light source, collimating or beam shaping optics may optionally be provided at the output of the optical fiber.

Light source 105 provides a substantially collimated beam of light to at least a portion of the sampling area 110. The intersection of the substantially collimated beam from light source 110 and the sampling area creates an interrogation zone, which is an illuminated portion of the sampling area. In one embodiment, where the fluid to be measured is air or

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some other gas, sampling area 110 is defined by the space between two air nozzles that provide air flow through the sampling area. In the embodiment of FIG. 1, sampling area 110 is defined by the space between two nozzles, an entrance nozzle 112, which supplies air to the sampling area, and an exit nozzle 114, which extracts air from the sampling area.

Upon illumination from light source 105, particles within sampling area 110 scatter light by Mie scattering. Mie scattering generally scatters light at angles inversely proportional to particle size. Accordingly, relatively small particles will scatter light at higher angles relative to the scattering produced by relatively larger particles. In practice, scattered light emerges from sampling area 110 in a cone centered about an axis defined by the collimated beam emerging from light source 105. The amount of light scattered into various angles is used, according to certain embodiments of the invention, to determine the size of the particles scattering light.

The system of FIG. 1 further includes a scattered light collection lens 115. In one embodiment, scattered light collection lens is a plano-convex lens arranged with the plano side facing toward sampling area 110 to minimize the spherical aberration associated with collecting and collimating scattered light. Scattered light collection lens 115 collects and collimates light scattered at relatively high angles by particles in sampling area 110 by being configured and positioned such that its front focal plane is co-incident with sampling area 110.

The system of FIG. 1 further includes a scattered light condenser lens 120. Scattered light condenser lens 120 takes collimated light emerging from scattered light collection lens 115 and focuses that light onto scattered light detector 125, which generates an electrical signal in proportion to the amount of scattered light incident on detector 125. In one embodiment, scattered light detector 125 is a photo-diode.

The system of FIG. 1 further includes beam blocking device 135. Beam blocking device 135 prevents further propagation of the collimated beam emitted by light source 105 after light emitted by light source 105 has propagated through sampling area 110. In one embodiment, beam blocking device 135 is a disk of optically absorptive material of a diameter somewhat greater than the beam diameter of the collimated beam emitted by light source 105. In the embodiment of FIG. 1, beam blocking device 135 is affixed to scattered light collection lens 115. In certain embodiments, beam blocking device 135 is a disc of black anodized aluminum. In other embodiments, beam blocking device 135 is a small light box with an absorptive interior coating arranged to force light emitted by light source 105 and entering the light box to undergo multiple internal reflections. Alternatively, beam blocking device is a fold mirror that directs light emitted by light source 105 to a beam dump arranged at some position outside of the optical components pictured. Alternatively, beam blocking device 135 is a conical shape with an absorptive interior coating that the beam enters at the open side. Beam blocking device 135 can be any device or combination of devices that prevents further propagation of the collimated beam, or stray reflections caused by the collimated beam, emitted by light source 105 after light emitted by light source 105 has propagated through sampling area 110.

The scattered light detection components of the system of FIG. 1 are arranged such that only a specific range of angles of scattered light are detected by scattered light detector 125. This can be accomplished in a number of ways. For example, the scattered light collection components can be sized and/or positioned in such a way as to only intercept light that has been scattered into a predetermined angular range of interest. Beam blocking device 135 inherently blocks very low angle

light, including the unscattered light that passes through the sampling area **110** from light source **105**. In one embodiment, beam blocking device **135** is sized to block not only light that is propagating along an optical axis defined by the beam from light source **105**, but also light that is scattered at low, but still non-zero angles. In this way, in certain embodiments, beam blocking device **135** is used to define a lower bound for the range of angles measured by the scattered light measurement components.

In certain embodiments, an upper bound for the range of angles measured by the scattered light measurement components is established by the position and size of scattered light collection lens **115**. Light scattered at high angles will not be intercepted by scattered light collection lens **115**, so the size and distance of scattered light collection lens from the plane of sampling area **110** defines an upper bound for the range of angles measured by the scattered light measurement components. Additionally or alternatively, annular masks of optically non-transmissive material may be placed in the scattered light detection path, for example, on scattered light condenser lens **120** or the scattered light collection lens **115** to limit the range of scattering angles measured.

The system of FIG. **1** further includes an ellipsoidal reflector **130**. The shape of ellipsoidal reflector **130** is defined with respect to a vertex located off the axis defined by the beam of substantially collimated light emitted by light source **105**. In other words, when viewed with respect to the axis defined by light source **105**, the collimated beam emitted by light source **105**, sampling area **110** and the scattered light collection components, ellipsoidal reflector **130** is an off-axis ellipse. A first focus of ellipsoidal reflector **130** is located at the sampling area **110** and is substantially co-incident with the front focal point of scattered light collector lens **115**. A second focus of ellipsoidal reflector **130** is located near the input port of a photomultiplier tube ("PMT") **150**, the function of which is set forth in further detail below. In one embodiment, ellipsoidal reflector **130** includes a circular aperture, for example, near its vertex, to allow for uninterrupted propagation of light from light source **105** to sampling area **110**.

Particles undergoing florescent emission within the sampling area **110** will emit light isotropically, that is, will emit equal optical power into all angles defining a sphere. Ellipsoidal reflector **130** is positioned such that it intercepts at least a portion of the light emitted by fluorescence from particles within the sampling area. The fluorescence light collected by parabolic reflector **130** at its first focus is directed along an axis defined by ellipsoidal reflector **130** toward the second focus located near an input port to PMT **150**.

The system of FIG. **1** further includes back-to-back long-pass optical filters **140**, i.e., two long-pass, reflective interference filters in series. In one embodiment, long-pass optical filters **140** are reflective interference type filters that transmit light having a wavelength longer than a certain wavelength while reflecting light having a wavelength shorter than a certain wavelength. The spectral characteristics of filters **140** are such that light emitted by particles within the sampling area **110** by fluorescence is transmitted, while light having substantially the same wavelength as that emitted by light source **105** is reflected.

Since fluorescence results in the emission of light having a longer wavelength than the excitation wavelength, filters **140** pass only light emitted by fluorescence, while reflecting noise (e.g., stray reflections) from the light source **105** as well as light scattered by particles within the sampling area **110** at angles toward PMT **150**. Two long-pass filters are used in series to improve the performance of the filters. This arrange-

ment is advantageous when placing the filters **140** in a converging beam, i.e., where the filters are used at non-zero angles of incidence.

The system of FIG. **1** further includes blower **160**. Blower **160** is arranged to draw fluid in the sampling area out of the system after it has been optically interrogated.

It is important to note that the specifics of the optical collection systems for scattered and fluorescent light described above with respect to FIG. **1** are exemplary and not required. Any combination or configuration of optical system capable of simultaneously collecting and measuring scattered and fluorescent light should be deemed to be within the scope of embodiments of the invention.

FIG. **2** shows the microbial collection components of a system according to an embodiment of the invention. In certain embodiments, the microbial collection components illustrated in FIG. **2** are used with the optical sizing and biological detection components described in reference to FIG. **1**. FIG. **2** omits the optical components for clarity, however. The system of FIG. **2** includes a housing **202**. In the system of FIG. **2**, air to be measured is routed to a sampling area **210** via an entrance nozzle **212**. Once in sampling area **210**, particles in the air are sized and classified as biological or non-biological by measuring the angular distribution of scattered light and fluorescence. One exemplary way this is accomplished by the operation of system components described above with respect to FIG. **1**. After being measured optically in sampling area **210**, air being measured is extracted from sampling area by exit nozzle **214** where it is passed to filter holder **260**.

Filter holder **260** contains microbe collection filter **265**. In one embodiment, microbe collection filter **265** is a 47 mm gelatin plate that can be removed from filter holder **260** and placed in a conventional Petri dish for incubation. Microbe collection filter **265** has a front side facing toward exit nozzle **214** and a rear side facing toward perforated support plate **266**, which itself includes perforations allowing fluid flowing through microbe collection filter **265** to flow the perforations into a rear chamber **267** of filter holder **260**. Rear chamber **267** is in fluid communication with exit fluid line **269**. Microbe collection filter **265** is supported by perforated support plate **266**, which includes a plurality of perforations allowing fluid to flow from the back side of the microbe collection filter **265** through the support plate **266**. Filter holder **260** is configured to provide an air-tight seal, by the use of compressed o-rings or the like, such that substantially all of the air extracted from sampling area **210** through exit nozzle **214** flows through the microbe collection filter **265**. From the back side of microbe collection filter **265**, air flows through the perforations in support plate **266**, and through fluid exit line **269**, which is in fluid communication with blower **270**. Blower **270** draws fluid through exit line **269**, the perforations in support plate **266**, and microbe collection filter **265**. Since microbe collection filter **265** and its support plate provide a partial obstruction to air flow, the suction created by blower **270** at the front side of microbe collection filter **265** will be less than the suction created by blower **270** near the back side of microbe collection filter **265**. The suction at the back side of microbe collection filter **265** is maintained at a level sufficient to extract the air being measured from sampling area **210**. This is accomplished by selecting sufficiently high blower suction, and a sufficiently high cross-sectional perforation area of the gelatin filter's support plate to maintain adequate negative pressure at the exit nozzle **214**.

In one embodiment, microbe collection filter **265** is a gas-permeable substance that causes microbial particles in fluid exposed to the filter to adhere to the filter. Additionally, microbe collection filter **265** is configured to maintain micro-

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bial viability so that microbes collected by the filter can be cultured and analyzed. In particular embodiments, microbe collection filter **265** is a gelatin wafer having a sufficiently high water content to maintain microbial viability, although the use of gelatin is not a requirement. Any filter containing a gas permeable substance with a high moisture content to maintain biological viability, to which microbes present in fluid exposed to the filter adhere, is acceptable to use as the filter described herein.

In the embodiment of FIG. 2, microbial collection filter **265** is removable so that, after a measurement period, the microbe collection filter **265** can be incubated and then analyzed to determine the number, and in certain embodiments, the types of microbes that were present in the measured air during the measurement period. In an exemplary process, after exposure, microbial collection filter **265** is removed and covered with a sterile cover for transport. After transport, microbial collection filter **265** is placed in contact with a growth medium containing plate, e.g., an agar containing Petri dish. The filter is then incubated for some amount of time, and the resulting microbial growth is analyzed. Analysis can take several forms. For example, microbial colonies may be visually visible and can be counted to determine the number of microbes that landed on the microbial collection filter during the measurement period. Additional analyses may be performed, for example, in certain cases the shape, i.e., the macroscopic appearance, of a microbial colony can provide information about the type of microbe present. Additionally or alternatively, microscopic observation of the microbes, chemical testing for the metabolic by-products of microbial growth and/or DNA analysis can determine the type of microbe collected.

FIG. 3 is an exploded drawing showing a microbe collection filter assembly according to an embodiment of the invention. FIG. 3 shows a preferred embodiment for the filter holder **260** described above with respect to FIG. 2. Microbe collection filter assembly includes cartridge housing **305**. Cartridge housing **305** includes hose barb **310** and flow sensor tube **315** which are arranged at sensor input aperture **320** on one side of the cartridge housing. This sensor assembly can be used to monitor air flow by the Venturi effect or the like. Fluid exiting the system enters cartridge housing, and therefore filter assembly, via input aperture **321** which is in fluid communication with an exit nozzle of the system, for example, exit nozzle **214** described above with FIG. 2. After being extracted from the sampling area of the system, fluid is routed into cartridge housing **305** via, for example, a non-illustrated hose attached to hose part **310**. Cartridge housing **305** also includes output aperture **322** and hose barb **325**. Fluid under test passes through output aperture **322** to non-illustrated blower after passing through microbe collection filter, which is described in additional detail below.

The assembly of FIG. 3 includes cartridge **330**, which includes a knurled handle for easier handling. The function of cartridge **330** is to hold a microbe collection filter in cartridge housing **305** such that the microbe collection filter is sealed in the flow path of fluid being extracted from the system. Cartridge **330** includes support plate **335**. Support plate **335** is, in one embodiment, a rigid disk containing perforations that allow fluid flow. An illustrative embodiment of support plate **335** is set forth in more detail below with respect to FIG. 4. Support plate **335** is secured to cartridge **330** by a fastener **340** that engages a through-hole in support plate **335**.

The assembly of FIG. 3 includes microbe collection filter **345**. In one embodiment, microbe collection filter is a disk of gas-permeable, water impregnated gelatin, but this is not a requirement. Microbe collection filter need only be capable of

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trapping some microbes present in air to which microbe collection filter **345** is exposed. Microbe collection filter is secured into cartridge **330** with o-ring **350** and clamp ring **355**. Once assembled cartridge **330** is inserted into and sealed against cartridge housing **305** with additional o-rings **360**, **365**. Cartridge **330** is secured in cartridge housing **305** using a plurality of pins **370**. Upon assembly microbe collection filter **345** is sealed in the fluid flow path emerging from the system such that all of the fluid emerging from the system is forced through microbe collection filter **345**.

FIG. 4 shows an exemplary filter support plate suitable for use in conjunction with an embodiment of the invention. In one embodiment, filter support plate is approximately 47 mm in diameter. The filter support plate of FIG. 4 includes a central aperture **405** having a diameter of approximately 3 mm. Central aperture **405** is optionally chamfered to receive a non-illustrated fastener. Filter support plate includes an outside annular zone **407**, configured to lay outside the area of microbe collection filter beings supported by the filter support plate. Outside annular zone **407** is optionally used for mounting or sealing to the filter support plate. In one embodiment, the filter support plate of FIG. 4 is approximately 1 mm thick.

The filter support plate of FIG. 4 includes a plurality of perforations **410** that allow air to flow from front to back. Although circular, radially arranged perforations are shown with respect to the filter support plate of FIG. 4, this not a requirement. The only requirement is that the filter support plate includes perforations sufficient to allow air to flow through a gas-permeable microbe collection filter, e.g., a gelatin wafer, in contact with the filter support plate while mechanically supporting the microbe collection filter against deformations caused by air pressure.

FIG. 5 shows a method of detecting and identifying microbes according to an embodiment of the invention. In the method of FIG. 5 particles in a fluid under test, for example air, are optically characterized. Optical characterization is conducted by illuminating the fluid under test with a beam of light, for example a beam of light generated by a laser or LED emitting at a wavelength of between 270 nm and 410 nm. Particles present in the fluid scatter light by Mie scattering at various angles depending on the size of the scattering particle. Additionally, biological particles in the fluid absorb light and re-radiate light at longer wavelengths by fluorescence. Light that is scattered into predetermined angles by particles in the fluid is detected and used to determine the size of the scattering particles. At the same time, light emitted by fluorescence from the particles is detected and used to make a determination as to whether the particles are biological or non-biological. The scattered light and fluorescence measurements are temporally correlated to determine the size of biological and non-biological particles present in the fluid being measured. Additionally, the scattered light and fluorescence measurements are stored, for example as a function of time, for later use and analysis.

After the fluid being measured is optically characterized, that fluid is exposed to a gas-permeable microbe collection filter, e.g., a gelatin wafer. After a predetermined period of time, the gas-permeable microbe collection filter is removed and cultured to encourage microbial growth. The culturing process comprises providing nutrients to microbes on the exposed gas-permeable microbe collection filter, and incubating same. After an incubation period has elapsed, the cultured microbe collection filter is analyzed. Such analysis can occur by any number of means, for example, colony counting, observing the macroscopic appearance of the growth patterns of microbes on the filter, microscope observation of the

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microbes, chemical testing for the metabolic by-products of microbial growth, or DNA testing.

If the analysis applied to the incubated filter determines the number of microbes that were present in the filter after the exposure period, this number is, in one embodiment, correlated with the optically measured data on the size and number of biological particles detected during the exposure period. Accordingly, correlating the optically measured data with the data from analysis of the cultured filter can be as simple as comparing the microbe count from the filter with the number of optically detected particles.

If the analysis applied to the incubated filter determines the types of microbes present on the filter at the time of exposure, this information, in one embodiment, is correlated with the stored data on the size and biological status of particles measured optically at the time the filter was exposed. This correlation optionally results in a determination of the number and types of microbes present in the fluid sample during the measurement period, i.e., the period of time the filter was exposed to fluid for which optical data was collected. This determination is made with the aid of conventional knowledge of the size of the types microbes identified on the filter by the analysis of the incubated filter.

The method illustrated in FIG. 5 has a number of advantages. First, the method of FIG. 5 provides a more complete retrospective characterization of the measured fluid sample by including information about the type of microbes that were instantaneously detected. Second, data collected according to the method of FIG. 5 allows for tentative, real-time characterization of future biological contamination events based on previously measured data. For example, suppose a contamination event occurs that results in numerous biological particles in the 0.4-0.8 μm range, which are instantaneously detected by the optical systems and methods set forth above. Upon analysis of a growth-medium containing filter exposed to air measured during this hypothetical contamination event, it is determined that most of these biological particles were a particular kind of microbe. In the future, whenever a spike of biological particles in the 0.4-0.8 μm range is detected under similar conditions, it can be assumed that another contamination event associated with the previously detected microbe is occurring in the monitored environment, and a real-time response appropriate to that contamination event can be coordinated. A predictive step is illustrated in FIG. 5, where microbial characteristics of a contamination event are predicted by matching optically collected data with previously optically collected data that was previously correlated to a particular set of identified microbes.

Computer hardware, software and machine vision components can be used to perform, assist or simplify and of the process steps performed herein. FIG. 6 shows an exemplary computer system for performing analysis of a fluid under test according to an embodiment of the invention. The system of FIG. 6 includes a particle sizing, biological particle identification, and microbial collection system 605. In certain embodiments, system 605 is the system described above with respect to FIGS. 1 and 2. System 605 performs real-time particle detection and sizing as well as real-time determination of whether detected particles are biological or non-biological. Additionally, system 605 performs microbial collection by exposure of a microbe collection filter to the fluid being optically measured. The optically measured data is converted to electrical signals by electronics included with system 605, for example, by the drive electronics associated with a photo-diode measuring optical scattering and a PMT measuring fluorescence light. The electrical signals generated by these two detectors are transmitted from system 605

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to optional computer data acquisition card 610, which is electronic communication with computer 615. Optically measured data is then stored in a persistent storage medium, for example, hard disk 620.

The system of FIG. 6 further includes machine vision camera 630, which is electronic communication with image capture board 625, which in turn is in electronic communication with computer 615. After a microbe collection filter 635 has been incubated, and microbial colonies are visible, camera 630 captures an image of the colonies visible on filter 635. To assist in this task, optional non-illustrated steppers may be used to translate the field of view of camera 630 with respect to filter 635 or vice-versa. An image or images of the filter showing microbial colonies is stored by computer 615 to disk 620.

Disk 620 also includes computer readable instructions 622 operable to cause computer 615 to correlate and/or compare the colony count detected by camera 630 with the optically measured data regarding the number and biological or non-biological status of particles measured during the time period when filter 635 was exposed. More generally, embodiments of the invention include instructions, such as instructions 622, residing in computer readable medium, such as for example computer hard drive 620 wherein those instructions are executed by a processor, such as processor residing in computer 615, to perform one or more of steps illustrated with respect to FIG. 5, for example the storage step, analyze step, correlate step or predict step. Computer readable instructions 622 need not reside on hard disk 620, but may reside in any medium capable of being in electronic communication with a processor capable of executing instructions 622. For example, there is no requirement that data (e.g., optically measured particle data) be stored on the same medium that includes instructions 622. Additionally, while FIG. 6 shows computer 620, camera 630 and other components being separate from system 605, this is not a requirement. System 605 could be configured to include a microprocessor, storage, memory, input/output electronics and a camera necessary to perform the method steps described herein.

In other embodiments, Applicants' invention includes instructions residing in any other computer program product, where those instructions are executed by a computer external to, or internal to, systems such as system 605, to perform one or more steps described with respect to FIG. 5. In either case, the instructions may be encoded in computer readable medium comprising, for example, a magnetic information storage medium, an optical information storage medium, an electronic information storage medium, and the like. By "electronic storage media," Applicants mean, for example and without limitation, one or more devices, such as and without limitation, a PROM, EPROM, EEPROM, Flash PROM, compactflash, smartmedia, and the like.

While the preferred embodiments of the present invention have been illustrated in detail, it should be apparent that modifications and adaptations to those embodiments may occur to one skilled in the art without departing from the scope of the present invention as set forth in the following claims.

What is claimed is:

1. A microbial detection and identification system comprising:
 - a sampling area defined by a fluid input nozzle and a fluid exit nozzle, the sampling area including a fluid;
 - a light source;
 - a first optical detector;
 - a second optical detector;

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a microbe collection filter in fluid communication with said sampling area, wherein the microbe collection filter has a front side facing the exit nozzle, and a rear side in fluid communication with a blower, which is adapted to supply sufficient negative pressure on the rear side of the microbe collection filter to evacuate the fluid from said sampling area as it is optically measured;
 wherein, said light source illuminates particles in said sampling area,
 said first detector detects light scattered into a predetermined angular range by particles of a predetermined size,
 said second detector measures light emitted by fluorescence from illuminated biological particles in said sample area,
 and said fluid is exposed to said microbe collection filter causing particles in said fluid to adhere to said microbe collection filter.

2. The system of claim 1, further comprising scattered light collection components that direct scattered light from said sampling area to said first detector and a plurality of optical components that direct fluorescence light from said sampling area to said second detector.

3. The system of claim 2, wherein said plurality of optical components includes a long-pass filter that selectively transmits light having a wavelength of light emitted by fluorescence by illuminated particles in said sample area.

4. The system of claim 3, further comprising an ellipsoidal reflector having a first focus at said sampling area and a second focus near said second detector.

5. The system of claim 4, wherein said long-pass optical filter comprises two long-pass reflective filters in series.

6. The system of claim 1, wherein said light source comprises an LED or a diode laser, and wherein said light source emits at a wavelength of approximately between 350 nm and 410 nm.

7. The system of claim 1, wherein said microbe collection filter includes a gas permeable interior allowing said fluid to flow into said front side, and out of said rear side.

8. The system of claim 1, wherein said microbe collection filter comprises gelatin.

9. A method of detecting microbial contamination in a fluid comprising:

providing a flow of fluid to be measured;
 illuminating a fluid with a light source;
 detecting particles of a pre-determined size range present in said fluid by measuring light scattered by illuminated particles into a predetermined range of angles;
 classifying particles in said fluid as biological or non-biological by measuring fluorescent light emitted from illuminated particles;
 providing a microbe collection filter with a front side arranged transverse to the flow of fluid to be measured;
 exposing the microbe collection filter to said fluid; and
 collecting particles on a microbe collection filter.

10. The method of claim 9, further comprising storing data related to the detection of particles of a predetermined size range and classification of particles as biological or non-biological for further analysis.

11. The method of claim 8, further comprising culturing said exposed microbe collection filter.

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12. The method of claim 11, further comprising analyzing the cultured exposed microbe collection filter to determine the types of microbes present on the microbe collection filter at the time of exposure.

13. The method of claim 12, wherein analyzing the cultured exposed microbe collection filter comprises one or more of: colony counting, observing the macroscopic appearance of the growth patterns of microbes on said cultured exposed microbe collection filter, microscope observation of microbes on cultured exposed microbe collection filter, and chemical testing for the metabolic by-products of microbial growth present on said cultured exposed microbe collection filter.

14. The method of claim 12, further comprising storing data related to the detection of particles of a predetermined size range and classification of particles as biological or non-biological for further analysis.

15. The method of claim 14, further comprising correlating said determination of the types of microbes present on the microbe collection filter at the time of exposure with stored data related to size of particles present in the fluid at the time of exposure and the status of said particles as biological or non-biological.

16. A method of characterizing a contamination event, comprising:

providing a flow of a first fluid to be measured;
 illuminating the first fluid with a light source;
 detecting particles of a pre-determined size range present in said first fluid by measuring light scattered by illuminated particles into a predetermined range of angles;
 classifying particles in said first fluid as biological or non-biological by measuring fluorescent light emitted from illuminated particles;
 storing data related to a scattering and fluorescence characteristics of particles in said first fluid;
 providing a microbe collection filter with a front side arranged transverse to the flow of the first fluid to be measured;
 exposing the microbe collection filter to said first fluid;
 collecting particles from the first fluid on the microbe collection filter;
 culturing said microbe collection filter;
 analyzing the cultured exposed microbe collection filter to determine the types of microbes present on the microbe collection filter at the time of exposure;
 correlating said determination of the types of microbes present on the microbe collection filter at the time of exposure with stored data related to the scattering and fluorescence characteristics of particles in said first fluid;
 optically detecting scattering and fluorescence characteristics of particles in a second fluid; and
 comparing the detected scattering and fluorescence characteristics in said second fluid with the stored data related to the scattering and fluorescence characteristics of particles in said first fluid.

17. The method of claim 16, further comprising, on the basis of said comparison, determining that microbes present in said first fluid are likely present in said second fluid.

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